Original Article

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Utility of Automated Hematology Analyzers in Diagnosis of Infective Etiologies: An Adjunct to Conventional Diagnostic Tools

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ABSTRACT

Background: Febrile illnesses such as malaria and dengue are challenging to differentiate clinically. For more than a century, the diagnostic approach is by clinical features and microscopy or manual immune-chromatographic tests. Automated cellular (WBC) indices from hematology analyzers may afford a preliminary rapid distinction. Several abnormal scattergrams during routine CBC analysis appear for the malaria and dengue.

Material and Methods: The present study is an observational, prospective study undertaken to evaluate the diagnostic utility of Sysmex XN-L series hematology analyzer as a screening tool to trigger specific testing for malaria and dengue. It includes patients suffering from either of the two febrile illnesses (malaria or dengue) at Hematology and microbiology section of Central Diagnostic Laboratory of a tertiary care centre in Central Gujarat. The sensitivity, specificity, PPV and NPV was performed for each abnormal pattern noted on scattergram for malaria and/or dengue. Interobserver variation is also taken in consideration.

Results: Malaria cases showed a significant statistical association with the presence of an extended neutrophil zone and dengue positive cases with NS1 antigen, IgG and IgM antibodies testing showed significant association with the hourglass pattern. Higher degree of agreement was noted for the hourglass pattern, doubling of neutrophil zone pattern among the observers

Conclusion: Significant statistical correlation was observed for extended neutrophil zone and malaria cases, hourglass pattern and dengue cases with a good agreement among the observers.

Keywords: *Automated Analyzer, White Blood Cell, Scattergram, Malaria, Dengue*

Introduction

Acute undifferentiated febrile (AUFI) illness is defined as acute onset of fever with temperature of >38°C of less than two weeks duration without an obvious cause despite a meticulous history and physical examination. In developing countries, differential diagnosis for AUFI includes some significant illnesses, such as malaria, dengue fever, bacterial sepsis, enteric fever, leptospirosis and rickettsiosis etc. both in term of mortality and morbidity [1].

Malaria is a common parasitic disease, major public health problem worldwide and a major cause of death in tropical and sub-tropical countries. 90% of fatalities occur in children of underdeveloped nations [2]. An estimated 50 million dengue infection occur annually and approximately 2.5 billion people live in dengue endemic countries [3]. In India dengue has seen a resurgence in recent times. Reported cases fatality rates in India are 3-5 %.

For diagnosis of malaria all suspected cases of malaria should have a parasitological test [microscopy or Rapid diagnostic test (RDT)] to confirm the diagnosis. Both microscopy and RDTs should be supported by a quality assurance programme [4]. The microscopic method is cost effective with a high degree of subjectivity involved in reporting of stained smears. Correct interpretation of the blood film requires considerable expertise [5]. Diagnosis of Dengue, according to WHO, may be done by two predominant methodologies, which includes a) virological tests, that directly detect elements of the virus (RT-PCR and NS1 antigen) and b) serological tests, which detect human-derived immune components(IgG and IgM antibodies) that are produced in response to the virus [6].

Complete Blood Count (CBC) is one of the commonest laboratory tests that is requested for almost all the febrile cases. Currently, most of the five part/six part automated hematology analyzers work on the principle of flow cytometry where cells in a suspension scatter a beam of laser/white light to produce scattergrams. The normal scattergram in the differential plot comprises of five components: lymphocytes, monocytes, neutrophil, basophils, eosinophils and a space between the neutrophil and eosinophil populations [7]. Automated hematology analyzer can also quantitate new parameters including cells in the atypical lymphocyte area (AL), high-fluorescent

lymphocyte counts (HFLC), immature granulocytes (IG), and immature platelets (IPF).

There are a few common infective etiologies that bring about specific changes in the blood cells. Hemozoin pigments produced in malaria, engulfed by neutrophils in blood scatter the laser light, thus exhibiting several abnormal scattergrams during routine CBC analysis [6]. While analyzing a CBC scattergram, an event in the plot points towards probability of diagnosing malaria. Dengue is characterized by the increase in atypical lymphocyte, which creates an abnormal event on the scatter gram [8].

Aim And Objectives

The present study was undertaken to assess the diagnostic performance of specific scattergram patterns for malaria and dengue. The objectives of the study were to:

- To compare the association of specific scattergram patterns with malaria and dengue positivity.
- To evaluate the ease of their use in identification of the specific scattergram patterns associated with dengue and malaria.

Materials and Methods

The present study is a prospective observational study done at a tertiary care center in Central Gujarat during the period of April 2019 to August 2020. The study was designed to use the scattergram generated by the automated hematology analyzer as a screening tool for malaria and dengue.

Febrile patients of all ages and both genders with a clinical suspicion of malaria or dengue were included in the study. For the selection of positive as well negative cases, the group of cases included in the study was given to a statistician for simple randomization. The randomized cases were included in study to decrease the bias done by the observer. Samples of non-febrile patients were excluded from the study.

Total 400 cases were taken with 20 positive cases of malaria and 150 positive cases of dengue. Malaria include 1:4 ratio of positive and negative cases, while for dengue 1:1 ratio for positive and negative cases were used.

The thick smears for detection of malaria parasite thick smears were prepared. To identify the species of malarial parasite thin smears were prepared. Thick smears were examined under oil immersion and number of parasite seen in oil immersion field as grading of load of parasitemia was reported. For dengue detection three rapid diagnostic methods suggested by WHO diagnostic criteria were used: NS1 antigen detection, IgG antibody detection, IgM antibody detection.

For complete blood count assessment samples were collected in EDTA vacuette and they were subjected to Sysmex XN series 350/550 automated hematology analyzer. In the present study, useful parameters included, according to study which were: Total Leucocyte Count, Haemoglobin, Platelet count and Differential count variables. All samples that were subjected to automated hematology analyzer for Complete Blood Count analysis were studied for scattergram patterns. In a normal scattergram five parameters were identified: Neutrophils + Basophils (Blue zone), Lymphocytes (Pink zone), Monocyte (Green zone), Eosinophils (Red zone) and Dark blue zone in bottom as debris.

Abnormal scattergram pattern were analyzer and classified as: **(Figure -1 & Figure -2)**

- Extended neutrophil zone
- Doubling of neutrophil zone
- Mixed pattern
- Hourglass pattern
- Other patterns: Grey zone, Eosinophilia and Monocytosis.

In the present study three observers have analyzed the images fetched from the scattergram data. Observer 2 and observer 3 were given single blinded data by observer 1. Agreement and diversity for each abnormal scattergram pattern was analyzed. The inter-observer variation has been taken for statistical analysis using kappa statistics. The sensitivity, specificity, PPV and NPV was performed for each abnormal pattern noted on scattergram for malaria and/or dengue.

Results

The study comprised of total four hundred (400) cases. Out of which, hundred (100) cases were of malaria with twenty (20) positive and eighty (80) control cases. Three hundred (300) cases were of dengue with one hundred and fifty (150) positive cases and the same number (150) cases of control group which were included in the study.

Demographic Profile of patients: In the present study, males outnumbered females as out of 400 cases 247 were male patients and 153 cases were female patients with a male: female ratio of 1.6: 1. Population of 1month to 84 years of age were included. Most of the malaria positive cases were seen in 20-29 years of age group while dengue positive cases were seen in 10-19 years of age group.

Clinical Presentation

The cases of malaria showed a significant statistical association with both the parameters of clinical

presentation: Temperature as well as splenomegaly. For dengue cases, significant correlation was seen between fever and NS1 antigen detection in dengue positive cases **(Table -1).**

All the samples were additionally run on a Sysmex XN series automated hematology analyzer (350 or 550) and studied for complete blood counts.

Correlative analysis of Hemoglobin, Total counts and Platelet counts with cases of Malaria and Dengue.

Decrease in total counts showed significant statistical association with NS1 antigen positive dengue cases, whereas decrease in platelet count was significantly associated with malaria and NS1 antigen positive dengue cases. Hemoglobin values showed little variation in values of positive negative cases, but there was no significant statistical association was established.

In Comparison of conventional method for malaria detection with scattergram patterns, malaria positivity showed significant statistical association with only 'Extended neutrophil zone' pattern of scattergram and no association was observed with any other scattergram pattern **(Table -2).**

In dengue, cases of NS1 antigen, IgG and IgM antibody positivity showed significant association with 'Hourglass pattern' of scattergram **(Table - 2).**

For Statistical analysis of scattergram patterns in cases of malaria, 'Extended neutrophil zone' pattern showed higher sensitivity and NPV values. High values of specificity and PPV was observed with 'Hourglass' pattern **(Table - 3).**

For dengue, 'Hourglass' pattern showed highest sensitivity and NPV for dengue cases of NS1 antigen and IgG and IgM antibody. While highest specificity and PPV was observed with 'Mixed pattern' and 'Doubling of neutrophil zone' pattern respectively for dengue cases of NS1 antigen. 'Mixed pattern' showed higher specificity and "Hourglass' pattern showed higher PPV for dengue cases with IgM antibody and higher specificity and PPV of 'Hourglass' pattern was observed for dengue cases of IgG antibody **(Table - 3).**

Inter-observer variation:

Observer number 1 and 2 showed a higher degree of agreement for hourglass pattern (k-0.55) followed by doubling of neutrophil zone (k-0.50), extension of neutrophil zone (k-0.45). While observer number 1 and 3 showed a higher degree of agreement (k-0.79) for doubling of neutrophil zone followed by extension of neutrophil zone (k-0.68) and hourglass pattern (k-0.66). Observer number 2 and 3 showed a higher degree of agreement for hourglass pattern (k-0.60), doubling of neutrophil zone (k-0.53) and extension of neutrophil zone (k-0.52).

Discussion

The present study is a prospective study of four hundred samples that included 400 cases, out of which 20 cases were malaria positive while 80 cases were malaria negative and 150 cases were Dengue positive while 150 cases were Dengue negative. This study was primarily undertaken to ascertain the Sensitivity, Specificity, PPV and NPV of each scattergram pattern generated for Malaria and Dengue cases. An additional inter observer variation has been added to the study to minimize the bias and interpretation error.

Demographic Profile of patients:

In the present study, population belonging to the age group of 1month to 84 years were included. Most of the malaria positive cases were seen in the 20-29 years age group while dengue positive cases were seen in 10-19 years age group. Males outnumbered females as out of 400 cases 247 were cases of male patients and 153 cases were female patients with a male: female ratio of 1.6: 1. In malaria positive cases out of 20 malaria positive cases 16 were male and 4 were female. Analysis of present study was consistent with Arti Negi et al. 2018[9] in which male: female ratio was 1.8:1.

Scattergram patterns:

In the study done by Arti Negi et al. (2018) [9] and Huh et al. (2008) [10] only malaria cases were included. On the contrary, in the present study we have included both malaria and dengue cases **(Table - 4).**

Scattergram patterns:

Extended neutrophil zone was the commonest pattern observed (90.0%) followed by doubling of neutrophils zone (30.0%) that is shown in **(Table 5)**. While contrast to other studies no normal scattergram was observed in malaria positive cases. Hour glass pattern and mixed pattern in present study were observed in 4 (20.0 %) and 2 (10.0%) cases.

In the present study, the most common pattern observed in dengue positive cases was the hour glass pattern in all the three gold standard methods (NS1 antigen, IgG antibody and IgM antibody) followed by extension of neutrophil zone, doubling of neutrophil zone and mixed pattern **(Table - 5).**

In the present study highest sensitivity and NPV of hour glass pattern was observed with dengue cases of NS1 antigen, IgG and IgM antibody. Mixed pattern showed higher specificity for NS1 antigen and IgM antibody while Hourglass pattern showed higher specificity and PPV for IgM antibody cases of dengue. The findings are compared with similar studies **(Table -6).**

Table 1: Correlation of malaria and dengue cases with clinical presentation.

	Absent		Clinical presentation – Temperature			Clinical presentation – Splenomegaly			Total
			Present	P value	Absent	Present	P value		
Malaria Positive		Negative	**75.0% (60)**	25.0% (20)	<0.001 55.00% (11)	97.50% (78)	**2.50% (2)**	<0.001 20	80
		20.0% (4)	**80.0% (16)**			45.0% (9)			
Dengue	NS1	Negative	**46.91% (77)**	53.09% (86)	<0.001	97.53% (159)	**4.27% (10)**	0.380	163
		Positive	**10.95% (15)**	89.05% (122)		99.27% (136)	**0.73% (1)**		137
	IgG	Negative	**31.45% (89)**	68.55% (194)	0.288	98.59% (179)	**1.41% (4)**	0.255	283
		Positive	**17.65% (3)**	82.35% (14)		94.12% (16)	**5.88% (1)**		17
	IgM	Negative	**31.49% (91)**	68.51% (198)	0.182	98.27% (284)	**1.73% (5)**	1.000	289
		Positive	**9.09% (1)**	90.91% (10)		100% (11)	**0**		11

[Chi square test]

Table 2: Comparison of conventional method for malaria detection and dengue with scattergram patterns.

		Extended neutrophil zone		Doubling of neutrophil zone		Mixed pattern		Hour glass pattern		
		Absent	Present	Absent	Present	Absent	Present	Absent	Present	Total
Malaria	Negative	83.75% (67)	**16.25% (13)**	95.0% (76)	**5.0% (4)**	97.50% (78)	**2.50% (2)**	98.75% (79)	**1.25% (1)**	80
	Positive	10.0% (2)	**90.0% (18)**	70.0% (14)	**30.0% (6)**	90.00% (18)	**10.00% (2)**	80.0% (16)	**20.00% (4)**	20
P value		<0.001		0.004		0.178		0.005		
NS1	Negative	87.65% (143)	**12.35% (20)**	97.66% (159)	**2.34% (4)**	99.38% (161)	**0.62 % (2)**	80.86% (132)	**19.14% (31)**	163
	Positive	83.21% (114)	**16.79% (23)**	98.54% (135)	**1.46% (2)**	99.27% (136)	**0.73% (1)**	60.58% (83)	**39.42% (54)**	137
P value		0.322		0.595		1.000		<0.001		
IgG	Negative	85.87% (243)	**14.13% (40)**	99.29% (281)	**0.71 % (2)**	99.65% (282)	**0.35 % (1)**	74.91% (212)	**25.09% (71)**	283
	Positive	82.35% (14)	**17.65% (3)**	94.12% (16)	**5.88% (1)**	94.12% (16)	**5.88% (1)**	11.76% (2)	**88.24% (15)**	17
P value		0.720		0.161		0.110		<0.001		
IgM	Negative	85.47% (247)	**14.53% (42)**	98.96% (286)	**1.04% (3)**	99.31% (287)	**0.69% (2)**	73.7% (213)	**26.30% (76)**	289
	Positive	90.91% (10)	**9.09 % (1)**	100 % (11)	**0 %**	100% (11)	**0 %**	9.09% (1)	**90.10% (10)**	11
P value		1.000		1.000		1.000		<0.001		

[Chi square test]

Table 3: Statistical analysis of scattergram patterns in malaria and NS1, IgG and IgM dengue.

		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Malaria	Extension of neutrophil zone	90.00	83.75	58.06	97.10
	Doubling of neutrophil zone	30.00	95.00	60.00	84.44
	Mixed pattern	10.00	97.50	50.00	81.25
	Hour glass pattern	20.00	98.75	80.00	83.16
NS1	Extension of neutrophil zone	16.79	87.65	53.49	55.47
	Doubling of neutrophil zone	1.46	97.66	66.67	55.30

		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
NS1	Mixed pattern	0.73	99.38	50.00	54.21
	Hour glass pattern	39.42	80.86	63.53	61.21
IgM	Extension of neutrophil zone	9.09	85.47	2.33	96.11
	Doubling of neutrophil zone	---	98.96	---	96.30
	Mixed pattern	---	99.31	---	96.31
	Hour glass pattern	90.91	73.70	11.63	99.53
IgG	Extension of neutrophil zone	17.65	85.87	6.98	94.55
	Doubling of neutrophil zone	5.88	99.29	33.33	94.61
	Mixed pattern	5.88	99.65	50.0	94.63
	Hour glass pattern	88.24	74.91	17.44	99.07

[Sensitivity, Specificity, PPV and NPV]

Table 4: Comparative analysis of scattergram patterns evaluated in different studies.

Scattergram patterns evaluated in different studies								
	Extension of neutrophil zone	Doubling of neutrophil zone	Mixed pattern	Hourglass pattern	Grey zone	Eosinophilia	Mono cytosis	Blue zone
Arti Negi et al. (2018)[9]	√	√	---	---	√	√	---	√
Huh et al. (2008)[10]	√	√	---	---	---	√	---	---
Present study	√	√	√	√	√	√	√	---

Table 5: Comparative analysis of Types of Scattergram Patterns Seen in Malaria Positive Cases.

Abnormal pattern	Arti Negi et al. (2018)[9]	Huh et al. (2008)[10]	In present study
Malaria positive Cases	65	144	**20**
Extended neutrophil zone	39.2% (20)	27.77% (40)	**90.0% (18)**
Doubling of neutrophil zone	29.41% (15)	2.77% (04)	**30.0% (6)**
Grey zone	17.64% (9)	NA	**10.00 % (2)**
Doubling of eosinophil zone	3.92% (2)	21.52% (31)	**NA**
Normal pattern	21.53% (14)	47.99% (69)	**NA**

Table 6: Comparative analysis of predictive biomarkers analysis for malaria scattergram pattern.

		Positive case	Negative case	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Arti Negi et al.[9] (2018)		51	234	78.46	90	66.23	94.35
Huh et al., [10] (2008)		144	319	69.4	100	-	-
Yoo et al., (2010)[7]		413	1388	46.2	99.7	-	-
Present Study	**Malaria extension of neutrophil zone**	**31**	**69**	**90.00**	**83.75**	**58.06**	**97.10**
	Malaria doubling of neutrophil zone	**10**	**90**	**30.00**	**95.00**	**60.00**	**84.44**
	Malaria mixed pattern	**04**	**96**	**10.00**	**97.50**	**50.00**	**81.25**
	Malaria hour glass pattern	**05**	**95**	**20.00**	**98.75**	**80.00**	**83.16**
	Malaria grey zone	**03**	**97**	**10.00**	**98.75**	**66.67**	**81.44**

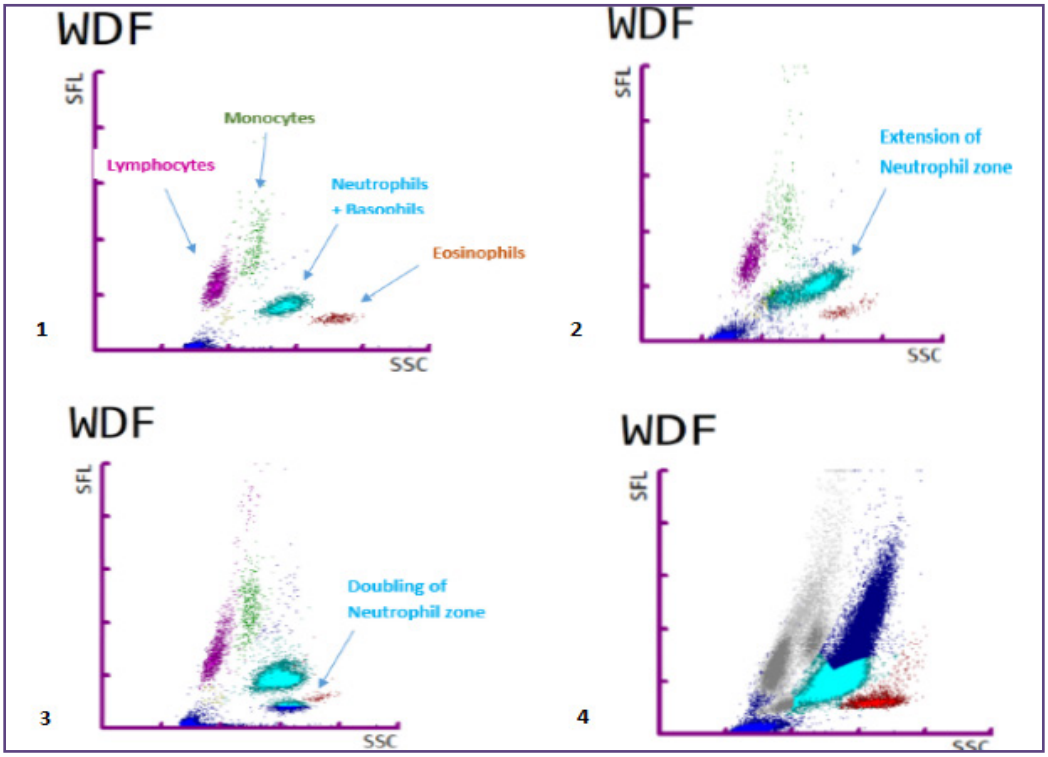

Fig. 1: 1- Normal scattergram, 2- Extended neutrophil zone scattergram, 3- Doubling of neutrophil zone scattergram, 4- Mixed pattern scattergram.

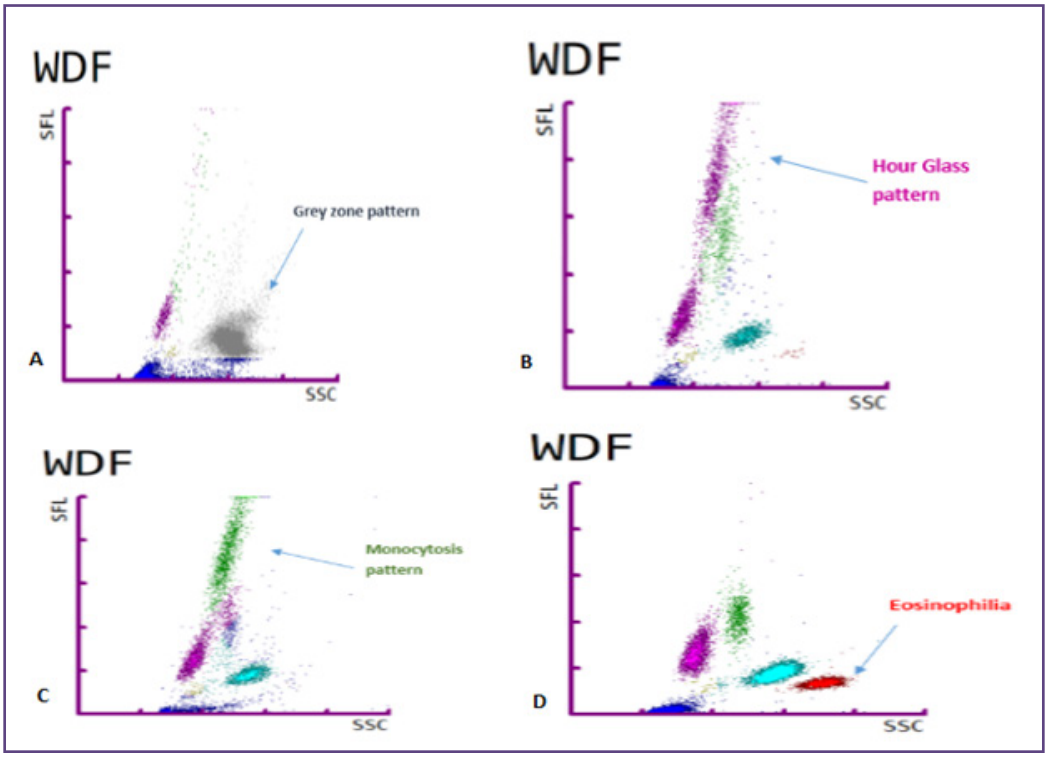

Fig. 2: A- Gray zone pattern scattergram, B- Hourglass pattern scattergram, C- Monocytosis pattern scattergram, D- Eosinophilia pattern scattergram.

Inter-observer variation:

Hourglass pattern and doubling of neutrophil zone pattern were observed having good degree of agreement among all observers.

Conclusion

'Extended Neutrophil Zone' pattern of scattergram has been observed to have a significant statistical association (p value<0.001) with the malarial smear positivity with higher sensitivity and NPV. Same as this, the 'Hour Glass' pattern of scattergram showed significant association with dengue serological positivity with higher sensitivity and NPV. 'Hour Glass' pattern and 'Doubling of Neutrophil Zone' pattern were easily identified by the technologists, without any extensive training required, as can be seen with the higher agreement among the three observers for the above mentioned patterns.

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Competing Interests

None

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